

Revision of USP General Chapter *Elastomeric Closures for Injection* <381>Dana M. Guazzo, PhD, *USP Parenteral Products–Industrial Expert Committee**

ABSTRACT The USP Parenteral Products–Industrial Expert Committee (PPI EC) has worked during the past three revision cycles to revise USP General Chapter *Elastomeric Closures for Injection* <381>. This *Stimuli* article summarizes these efforts and in the Appendix provides an overview of the current draft revision of <381>. USP <381> will be included in the *First Supplement to USP 31*, scheduled to become official on 01 August 2008. General Chapter <381> addresses the needs of the global pharmaceutical manufacturing environment by incorporating many of the tests contained in the *European Pharmacopoeia (Ph. Eur.) Rubber Closures for Containers for Aqueous Parenteral Preparations, for Powders, and for Freeze-dried Powders* 3.2.9 while retaining critical quality testing criteria in USP, such as *Biological Reactivity Tests*. Key modifications that appear in the revised <381> include:

1. *Establishment of Closure Classifications, Type I and II.* The revised chapter includes classification criteria for Type I and II closures.
2. *Addition of identification tests.* Unlike *Ph. Eur.* 3.2.9, <381> does not define specific identification tests. However, the chapter leaves it up to closure suppliers and end users to verify closure elastomeric formulations and coating materials according to suitable tests that meet pharmacopeial standards.
3. *Elimination of isopropyl alcohol (Extraction solvent C) and drug product vehicle (Extraction solvent B).* Water is the only extraction solvent specified in the revised chapter. To encourage the identification of extractables, the original chapter required additional solvents. The revised chapter's *Introduction* explains that <381> tests are intended as an initial screen. The end user is responsible for identifying and evaluating the safety of leachables in the packaged product.
4. *Elimination of closure sample preparation by autoclaving for 30 min.* The current chapter requires that closures be autoclaved for 30 min and then rinsed prior to the final extraction with the appropriate solvent. The revised <381> does not include this step. However, closures must conform to <381> both in their as-shipped state from the closure manufacturer and in their final ready-to-use state by the end user. Thus closures are required to meet <381> regardless of any prior processing to which they may have been exposed.
5. *Addition of specification limits for the following tests:*
 - Turbidity* [named *Appearance of Solution (Turbidity/Opalescence)* in revised <381>]
 - Reducing Agents* (named *Reducing Substances* in revised <381>)
 - pH Change* (named *Acidity or Alkalinity* in revised <381>)
 - Heavy Metals*.
6. *Addition of the following tests:*
 - Appearance of Solution (Color)*
 - Absorbance*
 - Extractable Zinc*
 - Ammonium*
 - Volatile Sulfides*
 - Functionality Tests:*
 - Penetrability*
 - Fragmentation*
 - Self-sealing Capacity*.
7. *Elimination of Total Extractables test.* This test (called the *Total Solids* test in *Ph. Eur.*) was eliminated in revised <381>. PPI EC determined that this test has limited value because of the typically low test results obtained with today's cleaner elastomeric formulations.

PPI EC concludes that revised <381> is suitable for compendial testing of closures intended for use in injectable product packaging.

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INTRODUCTION

The first efforts to revise *USP* <381> began during the 1995–2000 revision cycle. At that time many stakeholders believed that <381> should remain in its current format with the addition of test acceptance criteria/limits. Therefore the Parenteral Products–Industrial Expert Committee (PPI EC) began a lengthy process to obtain <381> test data to support the creation of meaningful acceptance criteria. Parenteral Drug Association (PDA) members who are experienced in pharmaceutical and closure manufacturing played a major role in coordinating this effort.

As time progressed, healthcare industries rapidly globalized, and product–package systems with closures that met the established specifications of the *European Pharmacopoeia* (*Ph. Eur.*) *Rubber Closures for Containers for Aqueous Parenteral Preparations, for Powders, and for Freeze-dried Powders* 3.2.9 became more cost effective. Therefore the PPI EC for the revision cycle 2000–2005 elected to create a completely revised <381> that would more closely mirror *Ph. Eur.* 3.2.9. The EC intended to reflect current industry trends and minimize the burdens of closure testing.

Creating an updated *USP* chapter that addressed closures for injectable products and was harmonized with *Ph. Eur.* proved to be a challenging task. PPI EC aimed to publish a revised *USP* chapter that would establish final tests that would be meaningful and useful for today's closure formulations and product–package systems. Numerous commenters suggested that *USP* simply copy the *Ph. Eur.* 3.2.9 chapter into *USP*. However, this would not allow users of *USP* to perform these tests because industry also would need access to many *Ph. Eur.* cross-referenced standards and test procedures that are not precisely duplicated in *USP*. Some recommended that *USP* should simply refer the reader to *Ph. Eur.* 3.2.9. But even referring a user of *USP* to *Ph. Eur.* would require the user to purchase the entire *Ph. Eur.*, and such an approach would not be consistent with *USP*'s general approaches to harmonization. Linking *Ph. Eur.* 3.2.9 to *USP* <381> and ensuring timely awareness of impending revisions to all the tests, procedures, and acceptance criteria, and cross-referenced standards and procedures would require that *USP* <381> be part of a more broadly based harmonization program. If <381> does enter such a program, the PPI EC expects that such efforts will have a greater likelihood of success because of the revisions that are now being made. PPI EC continued its efforts to create a *USP* <381> chapter that is useful to industry and also is aligned with *Ph. Eur.* 3.2.9 into the current 2005–2010 revision cycle.

In the course of the 2000–2005 revision cycle, the proposed *USP* <381> appeared in *Pharmacopeial Forum* (PF)29(1) 2003 and PF30(1) 2004. In response to numerous comments, PPI EC decided to verify certain physicochemical tests in an independent laboratory study. The EC did not consider this work necessary to justify the test procedures or the test limits (acceptance criteria) because these were already well established in *Ph. Eur.* Rather, this was an attempt to identify any technical problems associated with the new <381> proposed in PF 30(1) 2004, largely because of a) differing compositions of test solutions and b) because of different text formats and

wording between the compendia. Therefore, only one laboratory was chosen for the work. Some have commented that the PPI EC should have conducted a multi-laboratory collaborative study to generate statistically significant data. But the EC felt such an effort was unnecessary, would incur significant costs, and would cause unreasonable delays.

PPI EC selected Whitehouse Analytical Laboratories, LLC (WAL) (www.whitehouselabs.com) as the independent test laboratory for the study. This laboratory is GMP-certified and has undergone successful regulatory inspections. WAL's primary business is package component testing, and the company has expertise in both *USP* <381> and *Ph. Eur.* 3.2.9. These observations indicated that WAL was qualified for this work and, on this basis, was subcontracted by *USP* to perform the tests the PPI EC deemed necessary to advance <381> toward a significant revision. WAL performed this work at a much reduced rate as a service to *USP*.

At the request of *USP*, four suppliers provided closure samples: American Stelmi Corp., Helvoet Pharma, Hospira, Inc., and West Pharmaceutical Services, Inc. A total of 13 closure types were provided in a range of sizes, materials of construction, styles, and colors (*Table 1*). Upon receipt of the 13 closures a member of PPI EC blinded the sample set by assigning random identification numbers ranging from 1 to 13.

At the request of PPI EC, the closures supplied included those that the suppliers claimed could meet Type I as well as Type II specifications and even closures that would likely fail Type II specifications. Suppliers had the option to base their Type classifications on their own testing performed according to either *Ph. Eur.* 3.2.9 or the proposed specifications in new <381> in PF 30(1) 2004. How the suppliers defined *Type* is noted in subsequent data tables. Not all the samples submitted were marketed closures; rather PPI EC intended to use samples that represent a wide range of performance in order to thoroughly challenge the tests.

Table 2 lists the *Ph. Eur.* 3.2.9 physicochemical tests and their proposed *USP* counterpart tests performed at WAL. At WAL Analyst 1 tested each blinded closure population once according to *Ph. Eur.* 3.2.9 to provide an intralaboratory baseline for comparison. Then two analysts (Analyst 1 and Analyst 2) tested each population according to *USP* <381> for a total of two tests carried out according to the *USP* procedure. The *USP* <381> tests followed the text of PF 30(1) 2004 with certain editorial changes and corrections based on comments received. PPI EC compared these *USP* test results to the *Ph. Eur.* 3.2.9 data generated at WAL, as well as to relevant data provided by the suppliers. All raw data are retained by WAL, and copies are located at *USP* Headquarters.

After completion of the *USP*-sponsored research study, a representative of PPI EC presented an overview of the results from these tests, along with proposed *USP* <381> text, at the PDA 2007 Annual Meeting and invited comments. PPI EC also consulted the analysts at WAL regarding the General Chapter's textual clarity and accuracy. PPI EC relied on these comments and the research results and finalized *USP* <381>.

USP <381> RESPONSE AND RESEARCH RESULTS

The following section presents comments PPI EC received during the revision of <381>, EC responses, and data from the research study to show how the revision of USP <381> took place. Table 3 shows the general format of data tabulation. Tables 4–13 show all results from this study. Table 14 lists the final Type classifications for each closure sample determined from the research study results and compares this classification to the final Type classification for each closure as stated by the suppliers. The final text of USP <381>, as it will appear in the First Supplement to USP 31, appears in the Appendix.

1. Introduction

1.1 Comments. Numerous commenters requested clarification about the influence of coatings, laminations, and siliconization on compendial test requirements. Respondents also made requests about and suggestions regarding Type I vs. Type II closure definitions, differentiation, and test limits. Some suggested that Type II closures should not be reserved for multiple-dose products because current multi-dose products often are aqueous and should be packaged in Type I closures if possible. Other respondents asked USP to explain why a closure received a final classification of Type I instead of Type II. Finally, one commenter asked USP to delete the statement that closures should be sterilized before use in order to allow the use of clean but not sterile closures for products that are intended for terminal sterilization.

1.2 Response. PPI EC agreed to clarify closure types and materials of construction in USP <381>. The EC added specifics regarding closures made of silicone vs. those lubricated with silicone (e.g., dimethicone) and uncoated vs. laminated or lacquer-coated closures.

PPI EC agreed that the preferential use of Type I vs. Type II closures should be based on the aqueous vs. non-aqueous nature of the packaged preparation and not on the single-use vs. multiple-use functionality of the closure. USP <381> now states a general preference for Type I closures, but the Chapter notes that in some applications Type II closures may be more appropriate because of the extractables/leachables profile of the closure with specific products. Also, closures may not meet all the Type I requirements because of their physical configuration, material of construction, or both. PPI EC added a statement explaining that if a closure fails to meet even one of the Type I tests but meets the Type II requirements for that test, then the final classification of the closure is Type II.

Finally, the EC decided to retain the statement that closures should be sterilized before use in order to emphasize the critical nature of the injectable products being packaged. However, this statement is not intended to prevent the end user from performing technically and scientifically sound processes that are appropriate for specific products and that satisfy regulatory requirements.

2. Characteristics

2.1 Comments. Some suggested that this section could be deleted entirely because it is vague and offers no value. Another comment suggested improved and expanded wording.

2.2 Response. PPI EC decided to preserve this section and to adopt the proposed additional wording because it provides a general statement of quality expectations. Moreover, USP General Chapters are not intended to be prescriptive but rather serve to identify the principal issues relevant to meeting specifications.

3. Identification

3.1 Comments. The <381> text in PF 29(1) 2003 did not include the specific identification tests that can be found in Ph. Eur. 3.2.9. Respondents generally accepted this approach, and only one voiced a negative opinion. Some respondents made suggestions about more clearly and accurately describing the identification test examples in the General Chapter. One questioned the need for the closure supplier to perform identification tests at all and stated that this is the sole responsibility of the injectable product end user.

3.2 Response. PPI EC elected to adopt wording to improve and clarify the list of example identification tests provided. The EC agreed that the end user is responsible for identification testing, but the closure manufacturer should routinely provide identification test results to the end user in order to certify that the closures supplied match specifications.

4. Test Procedures

4.1 Comments. The <381> text in PF 30(1) 2004 suggested pretreatment of closures according to “actual use conditions” before compendial testing. Numerous respondents expressed concern that this deviates from Ph. Eur. 3.2.9, which allows, but does not require, pretreatment. Although EC members generally agreed on the importance of knowing if processing will cause closures to deteriorate and fail specifications, they felt that the present wording poses challenges to closure manufacturers who might not be in a position to determine what the relevant “pretreatment” should be. One respondent suggested that specific pretreatment processes be outlined in USP.

During the 2007 PDA Annual Meeting PPI EC proposed alternative wording that closures should meet requirements “both in their natural, manufactured state, as well as after processing.” Some commented that this may be confusing, e.g., at what point is a closure *natural*?; and if a closure manufacturer processes the components in some manner before shipment, are the components still *natural* after processing?; or should suppliers perform compendial tests both before and after such treatment? Instead, some commenters suggested that closures should comply with specifications both in their “as-shipped state” by the closure manufacturer and in their “ready-to-use state” by the end user. One respondent suggested that processing conditions should be documented only if such processing “deviates from the normal.”

An additional commenter, who was considering the relevance of physicochemical tests for laminated or coated closures, asked who should perform these tests if the latter are relevant only to the uncoated elastomer. Also, the respondent queried how firms should assess the impact of processing and sterilization in this case.

4.2 Response. In order to minimize confusion, PPI EC modified the text to state that closures must meet compendial specifications both when they are shipped from the closure manufacturer to the end user and in their final, ready-to-use state at the end user's facilities. Further explanation was added regarding processing/sterilization. If processing/sterilization are performed by the closure manufacturer before shipment, then the closures tested by the manufacturer should be so processed/sterilized before testing. Similarly, if the end user processes/sterilizes closures, the end user is responsible for testing closures exposed to sterilization processes.

Regarding laminated or coated closures, the EC added wording to clarify that physicochemical tests apply only to the uncoated base closures, which should be processed/sterilized according to the respective coated closure procedures before testing. The EC believes that the uncoated base closure should be tested and shown to conform to all <381> requirements even though the presence of the coating may act to improve the performance of the closure by minimizing extractables. Although the coating may lessen extractables, it should not be considered a complete barrier. Extracts from portions of the closure that are not coated may be deposited on the coated surfaces during closure processing, handling, or storage. Coatings may not be present on all surfaces that potentially may contact the contained product. And extracts may migrate through the coating to some extent during the product's shelf life.

If firms siliconize closures, they are not required to test closures after lubrication, especially if the presence of silicone may interfere with test results.

PPI EC does not agree with documenting only processing/sterilization conditions that fall outside the so-called norm because normal procedures may vary between closure types and over time.

5. Biological Tests

5.1 Comments. The majority of comments received related to the relevance of these tests in final closure classification and in relationship to *Ph. Eur.* harmonization. Questions submitted included: 1) which of the three *USP Biological Reactivity Tests, In Vitro* <87> should be applied; 2) why should Type I and Type II closures meet the same limits; and 3) why should biological safety tests be included—they are not part of *Ph. Eur.*?

5.2 Response. The final <381> text advises the reader to refer to *USP The Biocompatibility of Material Used in Drug Containers, Medical Devices, and Implants* <1031> for clarification regarding which *USP* <87> procedures should be performed. Type I and Type II closures must meet the same safety limits. As noted in the Introduction to *USP* <381>, these tests are intended only as an initial safety evaluation. Verification of the safety of closure leachables in the packaged product

must still be performed as part of a full package development process. With regard to harmonization, biological safety tests are not included in *Ph. Eur.*, but USP recognizes that closures approved for use in countries of the Council of Europe who have agreed to conform to the *Ph. Eur.* meet International Organization for Standardization (ISO) safety tests that are similar to those in <381>. Therefore the EC believes that including USP biological safety tests does not fall outside the spirit of harmonization.

6. Physicochemical Tests—Preparation of Solutions

6.1 Comments. The creation of *Solution S* using 200 mL of water per 100 cm² of closures according to *Ph. Eur.* 3.2.9 elicited several comments: 1) firms need an allowable surface area range; 2) analysts need to adjust the dilution volume in order to achieve an exact surface-area-to-volume ratio; 3) the procedure should allow rounding the dilution volume to the nearest 10 mL; and 5) the General Chapter should include procedures for standardizing the determination of surface area.

Respondents made the following comments regarding the heating process: 1) modify wording to “cover with boiling water”; 2) specify the times and temperatures for heating and cooling; and 3) permit other procedures for closing the container during heating.

Finally, respondents noted the need to describe the preparation of a *blank* solution.

6.2 Response. PPI EC paid a great deal of attention to this text. Clearly, any notable changes to the creation of the extract solution could affect all physicochemical test results and make *USP* <381> significantly different from *Ph. Eur.* 3.2.9. However, the EC acknowledges the ambiguity in the current *Ph. Eur.* dilution procedure, which specifies simply the use of 200 mL of water per 100 cm² of closures. For example, according to the *Ph. Eur.* procedure smaller closures of a given elastomeric formulation can yield different results than much larger closures of the same type because the actual surface area used for the uncut larger closures may deviate significantly from the target 100 cm². Other factors may enter into this difference in performance, but this error in surface-area-to-volume ratio alone is a significant cause for variation.

In the end PPI EC agreed to establish a range of 90–110 cm² for the target closure surface area. If the surface area lies within this range, 200 mL of water should be added, which is equivalent to *Ph. Eur.* 3.2.9. If it is not possible to achieve this target surface area with uncut closures, analysts are instructed to use the number of closures that most closely approximate 100 cm². In this case, the *USP* procedure calls for an adjustment in diluent volume to 2 mL of water per each 1 cm² of actual closure surface area used.

The USP research study required that *USP* <381> *Solution S* be prepared by addition of 2 mL of water for every 1 cm² of actual surface area used, but *Ph. Eur.* 3.2.9 stipulates that *Solution S* be prepared without dilution adjustment. Therefore, the research study test results represent worse-case differences between procedures for *Solution S* preparation.

PPI EC has no plans to add procedures for surface area calculation. The EC made no changes to the text regarding how the container should be closed, nor did the EC make any

changes to the heating/cooling steps, primarily in order to maintain consistency with *Ph. Eur.* The EC added steps for the preparation of a *blank* solution.

7. Appearance of Solution (Turbidity/Opaescence and Color)

7.1 Comments (Turbidity). Most respondents were in favor of establishing an instrumental procedure for turbidity. However, some expressed concern about the lack of an established relationship between the current *USP* <381> turbidity test based on visual appearance and the proposed instrumental procedure. One commenter suggested publication of a *Stimuli* article to explain and justify the use of the instrumental turbidity tests and the pass/fail criteria.

Regarding the turbidity Reference Standards, comments included: 1) the dilution volumes are incorrect for *Reference Suspension A*; 2) *Reference Suspension C* should be deleted; 3) water should be added as a reference; 4) Reference Standard formulation steps should be deleted, and the user should simply purchase calibrated standards; 5) the meaning of Fourier Turbidity Units (FTUs) should be explained; and 6) the turbidity specifications in *USP* should be justified because they are more strict than those in *Ph. Eur.*

Respondents also suggested that the test section title be modified and that subtitles should be added.

7.2 Comments (Color). Comments focused on an apparent error in the preparation of the *Color Standard*. Specifically, commenters suggested that *Solution S* should be not more intensely colored than a mixture of 3.0 mL of *Matching Fluid O* (rather than 6.0 mL) and 97.0 mL of diluted hydrochloric acid, viewed similarly (cf. *USP Color and Achromicity* <631>).

7.3 Response. PPI EC determined that the traditional *USP* text format and terminology in this section are particularly confusing and easily misinterpreted, especially because of the complexity of the tests. Therefore, the EC made several changes, including reorganizing the section with appropriate titles and subtitles. Simple-to-follow subsections spell out the creation of turbidity standards and reference solutions. These subsections match the format of *Ph. Eur.* 3.2.9. The EC added a table that clarifies the proper creation of turbidity *Reference Suspensions*, including their turbidity values. A second new table clearly defines the turbidity pass/fail criteria for Type I and Type II closures. The EC corrected errors in the dilution volumes of turbidity standards and test solution specification limits and added a note that allows the use of commercially available turbidity standards.

At the time of *PF* 30(1) 2004, *Ph. Eur.* 3.2.9 included only a visual inspection procedure for turbidity. Thus the EC based the proposed procedure on ISO 8871-1:2003(E). *Ph. Eur.* 3.2.9 has since been revised and also includes an instrumental procedure for turbidity determination based on ISO 8871-1, as cross-referenced in *Ph. Eur. Clarity and Degree of Opalescence of Liquids* 2.2.1. To ensure the comparability of *USP* and *Ph. Eur.*, *USP* has discontinued use of FTU units and has adopted Nephelometric Turbidity Units (NTUs). One FTU = 1 NTU. Although analysts do not need *Suspension D* to determine turbidity, the EC added it to conform further to *Ph. Eur.* 2.2.1.

Both *USP* and *Ph. Eur.* retain the visual procedure. Both compendia include a note that encourages the use of the instrumental procedure because of its greater discriminatory power and because it depends less on analysts' visual acuity.

PPI EC checked the directions for creating the *Color Standard* reported in *PF* 30(1) 2004 and found that they were correct (6.0 mL of *Matching Fluid O* diluted with 94 mL of dilute acid). The confusion is due a difference in *USP*'s *Matching Fluid O* and *Ph. Eur.*'s *Solution GY*. The concentration of *Solution GY* is twice that of *Matching Fluid O*. Therefore, although *Ph. Eur.* specifies the use of 3.0 mL of *Solution GY* diluted to create the final *Standard Solution GY5*, *USP* specifies 6.0 mL of *Matching Fluid O* diluted to create the final *Color Standard*.

7.4 Research Results Appearance of Solution (Turbidity/Opaescence). Tables 4 and 5 show the turbidity test results for *Procedure A (Visual)* and *Procedure B (Instrumental)*, respectively. These results indicate that almost all closures that suppliers expected to meet Type I specifications for this test did meet Type I criteria when tested by both visual and instrumental procedures. Closure 13 was an exception because Analyst 1 judged that this closure met Type II turbidity specifications (*USP* visual test).

Analysts might have more difficulty correctly identifying Type II closures according to the visual test. For example, both analysts evaluated Closure 9 as Type I, but the instrumental results clearly supported the supplier's Type II test-specific classification. Closure 12, which the supplier said was likely to fail Type II criteria, clearly demonstrated acceptable Type II results by the instrumental method, but visual readings yielded a result of Type I in 2 of 3 examinations.

Closure 8 results were conflicting because turbidity readings were remarkably different between Analyst 2 (7.7 NTU) and the first results from Analyst 1 (0.2 NTU). This difference also appeared in the visual test results. *USP* suspected the results indicated a laboratory error and requested that Analyst 1 repeat the visual and instrumental test readings for this closure. The repeat tests clearly indicated Type II readings instrumentally (9.4 NTU). The visual results were still conflicting: Type I according to *Ph. Eur.* and Type II according to *USP*.

Thus the turbidity test is not always a clear indicator of Type I vs. Type II classification, especially for Type II closures. Generally, the visual method proved reliable for Type I closures with a low levels of extractables. If *Solution S* was more than negligibly turbid, visual interpretation often led to error. The instrumental method was more reliable and allowed quantitative data analysis.

7.5 Research Results Appearance of Solution (Color). Table 6 shows the results from the color of solution analyses. All closures met the supplier's test-specific Type criteria when they were evaluated according to either the *Ph. Eur.* 3.2.9 procedure or the proposed *USP* <381> procedure.

8. Acidity or Alkalinity

8.1 Comments. Comments regarding the *Acidity and Alkalinity* test focused on the need to change the text to make it more user friendly and less subject to misinterpretation. For example, respondents said this section should clarify that only one

titration process should be conducted, and the titration should be based on the initial color of the *Solution S* plus *Bromothymol Blue Solution* mixture. Some commenters stated that pH titration tests require a blank titration according to *USP Titrimetry* (541) and requested a statement to this effect.

8.2 Response. PPI EC agreed to change the text to improve clarity and added a *blank* correction step as recommended in (541). The EC also added a “Requirements” statement to all (381) tests in order to clearly define the acceptance criteria.

8.3 Research Results. Table 7 shows the results from the *Acidity and Alkalinity* tests performed at WAL. The closure manufacturers stated that all samples should pass this test. All closures passed, with the exception of Closure 8 when tested by Analyst 1 according to *Ph. Eur.* 3.2.9. PPI EC asked Analyst 1 to repeat this test, and the repeat results passed specifications.

9. Absorbance

9.1 Comments. Several commenters noted that the absorbance spectrum range should be 220–360 nm rather than 200–360 nm as specified in *PF* 30(1) 2004. Commenters also suggested that the absorbance readings should be performed using the *blank* in the reference beam and that analysts should filter *Solution S* only if they observe turbidity.

9.2 Response. In the final text PPI EC corrected the error in the spectrum range that appeared in *PF* 30(1) 2004 and also agreed to the use of the *blank* rather than water. Because visual interpretation of turbidity yields results that are highly variable, the EC elected not to include a statement permitting filtration of *Solution S* only if it is visibly turbid. In order to further harmonize with *Ph. Eur.* 3.2.9 *Absorbance* text, PPI EC included instructions that “if dilution of the filtrate is required prior to measurement of the absorbance, correct the test results for the dilution.”

9.3 Research Results. Table 8 shows the *Absorbance* test results. In every case the WAL Type classification results conformed exactly to the test-specific Type classifications that the manufacturers provided. Importantly, the absorbance readings for all these blinded closure samples tested according to the *Ph. Eur.* 3.2.9 procedure closely matched the data generated according to proposed *USP* (381) procedure, as well as the supplier’s data. (Closure 8 data generated by Analyst 1 for other tests demonstrated apparent discrepancies. To ensure that Closure 8 absorbance readings generated by Analyst 1 were reliable, the EC requested that Analyst 1 repeat this test. Both Closure 8 absorbance data sets generated by Analyst 1 yielded similar results.)

10. Reducing Substances

10.1 Comments. Most respondents suggested modifications to ensure a closer match to *Ph. Eur.*: 1) Impose a 4-hour limit from the time of *Solution S* preparation to the performance of the test; 2) change the directions for solution cooling from

“cool rapidly to room temperature” to simply “cool”; and 3) describe the blank titration step more clearly in order to avoid error.

10.2 Response. PPI EC agreed with all comments and made changes accordingly. In addition, the *USP* procedure was changed to duplicate the *Ph. Eur.* procedure that requires the use of 0.01 M sodium thiosulfate rather than 0.01 M sodium thiosulfate VS. This change was made to prevent confusion because the current USP sodium thiosulfate VS solution is a 0.1 N buffered formulation.

10.3 Research Results. Table 9 presents the *Reducing Substances* test results. As observed in some other tests, the data generated at WAL according to either *Ph. Eur.* 3.2.9 or the proposed *USP* (381) consistently predicted the suppliers’ test-specific classification for those Type I closures with the lowest apparent extraction profiles (Closures 1, 3, 4, 5, 6, 7, 10, and 11). Within this group of closures, manufacturer-supplied data predicted Closures 1 and 11 would exhibit the greatest titration volume difference. This relative ranking was similar to the data generated by WAL.

The supplier of Closure 9 claimed that it conformed to Type I for this test, although the supplier’s titration volume difference was notably close to the specification limit. WAL data also indicated that the titration volumes approached and in one case exceeded the Type I limit (Analyst 2, *USP* method).

The manufacturers certified that Closures 2 and 12 conformed to Type II for this test, but the WAL data consistently supported a Type I classification for both closures, although titration volumes approached the Type I limit.

The supplier specified that Closure 8 was a Type II closure for this test. The WAL data also supported a Type II classification (according to both *USP* and *Ph. Eur.*), although the initial analysis by Analyst 1 supported a Type I classification (even though the data approached the Type I limit).

The supplier of Closure 13 specified that it met Type I specifications for this test. WAL data reflected both Type II (*USP/Ph. Eur.* Analyst 1) and Type I (*USP* Analyst 2) classifications. But WAL analysts recorded titration volume differences that were not remarkably different from the supplier’s anticipated volume.

Overall the WAL data generated according to the *USP Reducing Substances* (381) test were comparable to the intralaboratory results obtained according to *Ph. Eur.* 3.2.9.

11. Heavy Metals

11.1 Comments. Several respondents noted that the proposed *USP Heavy Metals—Method I* (231) test does not duplicate the *Ph. Eur. Heavy Metals Method A* 2.4.8 test. Respondents suggested that if the compendial procedures remain different, USP should conduct a detailed comparison of the two procedures. Otherwise, they requested, USP should incorporate the *Ph. Eur.* method in its entirety. Finally, one suggested that *USP* allow the user to follow either the *USP* (231) or the *Ph. Eur.* 2.4.8 test.

11.2 Response. PPI EC understands that the two compendial *Heavy Metals* tests are noticeably different, which can at times be challenging for industry. However, simply copying the *Ph. Eur. Heavy Metals* test with all its cross-referenced standards,

etc. into *USP* is unworkable because future revisions to these *Ph. Eur.* references would immediately negate harmonization. Further, *USP* (231) is being considered for future revisions, so conducting an exhaustive method comparison of the current *USP* vs. the current *Ph. Eur.* to demonstrate equivalence would be both time consuming and counterproductive. At present *USP* requires users to conduct a unique *Heavy Metals* test.

PPI EC decided to add to the procedure a statement explaining that analysts should prepare the *Test Preparation* for *USP Heavy Metals—Method I* (231) using 10.0 mL of *Solution S*. The *USP* Research Study showed that without this clarification analysts could easily make mistakes when they prepared the *Test Preparation*.

11.3 Research Results. Table 10 presents the *Heavy Metals* test results. All manufacturers' certifications simply stated that the closures should pass either the *USP* or *Ph. Eur. Heavy Metals* specifications but did not supply detailed data. All sample populations tested at WAL passed the specification criteria from both compendia.

12. Extractable Zinc

12.1 Comments. Commenters pointed out that *USP* text that appeared in *PF* 30(1) 2004 included the redundant addition of 0.5 mL of 0.1 N HCl for *Test Solution* preparation. They also pointed out that the text did not supply any specific directions regarding the preparation of a *blank*. Respondents suggested that the *Zinc Standard Solution* should be prepared to a final concentration that conforms to *Ph. Eur.* because otherwise the final limits would be affected. They pointed out that the zinc emission wavelength specified in the *PF* text was in error (it should be 213.9 nm, not 213.8 nm). Commenters suggested that the specification should be reworded so that it more closely reflects wording of *Ph. Eur.* ("maximum of 5 µg of extractable Zn per milliliter of *Solution S*"). They also pointed out that the *USP* procedure requires that the *Test Solution* be compared directly to the *Reference Solution*, which differs from *Ph. Eur.* and specifically requires the creation of a calibration curve. Finally, they requested that inductively coupled plasma (ICP) be allowed as an alternative analytical tool.

12.2 Response. PPI EC agreed to make the necessary changes regarding the preparation of the *Test Solution* and the *blank* and to incorporate the corrections to the *Zinc Standard Solution* and the zinc emission wavelength. During the *USP* research study PPI EC became aware that the *USP* text as it appeared in *PF* 30(1) 2004 was difficult to follow. The EC also acknowledged that use of a calibration curve as specified by *Ph. Eur.* is a preferable procedure compared to the draft *PF* approach. Therefore the EC reworded the text of this test and the specification using a format similar to that of *Ph. Eur.* 3.2.9 and incorporated additional text that is similar to that of *Ph. Eur. Atomic Absorption Spectrophotometry—Method I* 2.2.23. Finally, PPI EC added use of ICP as an alternative analytical tool.

12.3 Research Results. Table 11 presents the *Extractable Zinc* test results. All manufacturers' certifications stated, without supplying detailed data, that the closures should pass the *USP* and *Ph. Eur.* specification of no more than 5 ppm extrac-

table zinc for *Solution S*. All sample populations tested at WAL passed the *USP* and *Ph. Eur.* compendial specification limit.

13. Ammonium

13.1 Comments. Commenters pointed out that the concentrations of sodium hydroxide solution and alkaline mercuric potassium iodide in *USP* differ from those in *Ph. Eur.* They also noted that the wording used for solution preparation and test procedures sometimes was difficult to follow.

13.2 Response. PPI EC agreed to reword and reorganize this test, which now includes subsections that explain the preparation of the *Test Solution*, the *Alkaline Potassium Tetraiodomercurate Solution*, and the *Ammonium Standard Solution*. The EC intends that the preparation of these solutions and the test itself should parallel *Ph. Eur. Ammonium* 2.4.1.

13.3 Research Results. Table 12 shows the results of the *Ammonium* test performed at WAL. The manufacturers certify that all closure samples meet *USP* and *Ph. Eur.* specifications, which the test results confirm.

14. Total Solids

14.1 Comments. Commenters strongly recommended that this test be eliminated because current elastomeric formulations are much cleaner than those in the past and yield very low total solids results that are lower than the error associated with the test procedure itself. To support this argument, commenters provided data showing that *USP* tests for *Opalescence*, *Reducing Substances*, and *Absorbance* are more sensitive indicators of closure quality.

14.2 Response. PPI EC agreed to eliminate the *Total Solids* test.

15. Volatile Sulfides

15.1 Comments. Commenters made recommendations to modify the wording of this test in various ways, all of which generally moved away from traditional *USP* style and toward a format similar to that of *Ph. Eur.* Respondents also suggested that the surface area of the closures should match *Ph. Eur.* specifications exactly, including tolerances. Finally, one commenter recommended adding time constraints to the heating and exhaust portions of the autoclave cycle.

15.2 Response. PPI EC agreed to modify the wording generally in accordance with *Ph. Eur.* 3.2.9. The EC also revised the closure surface area as recommended to 20 ± 2 cm². The EC declined to add restrictions to the autoclave cycle and thus concurs with the cycle described in *Ph. Eur.* 3.2.9.

15.3 Research Results. Table 13 contains the results from the *Volatile Sulfides* test performed at WAL. All sample test results conformed to *USP/Ph. Eur.* specifications in agreement with the closure manufacturers' certifications.

16. USP <381> Physicochemical Tests—Conclusion

PPI EC used the physicochemical tests data generated at WAL during the USP research study to categorize each blinded closure sample according to a final Type classification. In doing so the EC applied the definition for “final classification” as stated in the final USP <381> text: “If a closure fails to meet one or more of the Type I test requirements but still meets the Type II requirements for the test(s), assign the closure a final classification of Type II.” Table 13 compares the final Type classifications according to WAL data and the supplier’s final classifications for each sample. The data sets according to both USP <381> and *Ph. Eur.* 3.2.9 correctly identified all Type I closures. The data sets correctly identified all Type II closures, with the exception of one *Ph. Eur. Acidity and Alkalinity Test* 3.2.9 data point for Closure 8. The supplier of Closure 12 noted that it was likely to fail Type II classification. However, the sample met Type II specifications when tested either according to *Ph. Eur.* 3.2.9 or USP <381>.

17. Functionality Tests

17.1 Comments. Several commenters recommended adding a requirement that closures be pretreated according to *Ph. Eur.* before functionality evaluation. The PF 30(1) 2004 text included a recommendation that analysts may perform additional functionality tests using needles that reflect actual conditions of product package use. Commenters generally agreed with this recommendation but expressed concerns that this procedure is different from that of *Ph. Eur.* and that the intent of this recommendation may not be clear. One respondent submitted an entirely new set of functionality tests that were designed to be more product/package specific.

17.2 Response. PPI EC agreed to state that closures should be pretreated as described for *Solution S* preparation and air dried before use. The EC agreed to remove the recommendation to consider other actual product/package needle combinations and, for the sake of standardization, to refer only to ISO standard 21-gauge needles. The EC agreed not to modify the functionality tests to make them more product specific. Instead the EC decided that the tests will remain identical to the *Ph. Eur.* 3.2.9 tests.

18. Penetrability

18.1 Comments. A few recommendations addressed *Penetrability*, including a suggestion to eliminate redundant text describing the needles to be used and to clarify that a new needle should be used for each closure.

18.2 Response. PPI EC adopted the suggestions.

19. Fragmentation

19.1 Comments. The Fragmentation test described in PF 30(1) 2004 included an aqueous test for Type I closures and a sesame oil test for Type II closures. All comments about the *Fragmentation* test referred in some way to this water vs. oil approach to testing closures. Commenters recommended that the text be made the same as that in *Ph. Eur.*, i.e., that closures intended for liquid preparations should be fitted onto vials containing water and that closures intended for dry preparations should be fitted onto empty vials.

19.2 Response. PPI EC agreed to change the text of this test to match *Ph. Eur.* 3.2.9.

20. Self-sealing Capacity

20.1 Comments. The PF 30(1) 2004 version of this test included a 0.5% methylene blue solution. Several commenters recommended that the dye concentration be 0.1% as is the case in *Ph. Eur.*

20.2 Response. PPI EC agreed to require the use of a 0.1% methylene blue dye.

ACKNOWLEDGMENTS

PPI EC wishes to thank the closure manufacturing industry, the pharmaceutical industry, the PDA Packaging Science Subcommittee, and WAL for their support of revision of USP <381>. Responses to PPI EC’s requests for comments during the current and previous revision cycles were thoughtful and merited careful consideration. Since the appearance of <381> in PF 29(1) 2003, USP received 44 letters that included 240 separate comments. These comments often included data reflecting many hours of effort. PPI EC tabulated each comment and carefully reviewed it multiple times, making the new USP <381> a concerted effort of USP and end users.

Table 1. Closure Test Sample Descriptions

Closure sample types	13 variations	
Elastomers	Butyl, bromobutyl, styrene butadiene, EPDM, blend, others not specified	
Closure sizes	8, 13, 20, 28, 32 mm	
Closure surface areas ¹	Ranging from 4.6 to 30.6 cm ²	
Closure styles	Plug, lyophilization, spike, syringe plunger	
Closure colors	Red, light to dark gray, black	
Closure coatings	Not applicable	
Meets Type I final classification	8 samples (total)	
	6 samples	according to <i>Ph. Eur.</i> 3.2.9
	2 samples	according to <i>USP</i> <381> in <i>PF</i> 30(1) 2004
Meets Type II final classification	4 samples (total)	
	3 samples ²	according to <i>Ph. Eur.</i> 3.2.9
	1 sample	according to <i>USP</i> <381> in <i>PF</i> 30(1) 2004
Fails Type II final classification	1 sample (total)	
	1 sample	according to <i>Ph. Eur.</i> 3.2.9

¹ Surface areas were determined by the suppliers.

² One sample may not meet Type II according to the supplier.

Table 2. USP Closure Research Study Tests Performed

<i>USP</i> <381> ¹	<i>Ph. Eur.</i> 3.2.9
<i>Appearance of Solution, Turbidity (Opalescence) Procedure A</i>	<i>Appearance of Solution, Turbidity</i>
<i>Appearance of Solution, Turbidity (Opalescence) Procedure B</i>	n/a ²
<i>Appearance of Solution, Determination of Color</i>	<i>Appearance of Solution, Color</i>
<i>Acidity or Alkalinity</i>	<i>Acidity or Alkalinity</i>
<i>Absorbance</i>	<i>Absorbance</i>
<i>Reducing Substances</i>	<i>Reducing Substances</i>
<i>Heavy Metals</i>	<i>Extractable Heavy Metals</i>
<i>Extractable Zinc</i>	<i>Extractable Zinc</i>
<i>Ammonium</i>	<i>Ammonium</i>
<i>Volatile Sulfides</i>	<i>Volatile Sulphides</i>

¹ *USP* <381> tests were performed as described in *PF* 30(1) 2004 with changes incorporated according to comments received.

² The current *Ph. Eur.* *Appearance of Solution, Turbidity* 3.2.9 test utilizing the instrumental turbidimetry method of *Ph. Eur.* 2.2.1 was not part of *Ph. Eur.* at the time the study was performed (n/a).

Table 3. Name of *USP* <381> Test—*EXAMPLE ONLY*

Specification					
Closure Random Identification Number	Closure Manufacturer–Supplied Information ¹		WAL Test Results		
	Final Type Classification	Test-Specific Classification	<i>Ph. Eur.</i> Analyst 1	<i>USP</i> Analyst 1	<i>USP</i> Analyst 2
1	I	I			
3	I	I			
4	I	I			
6	I	I			
7	I	I			
10	I	I			
5	<i>I</i> _{USP} ¹	<i>I</i> _{USP}			
11	<i>I</i> _{USP}	<i>I</i> _{USP}			
2	II	I			
8	II	II			
9	<i>II</i> _{USP}	<i>II</i> _{USP}			
13	<i>II</i> _{USP} ²	<i>I</i> _{USP}			
12	Fail II	Fail II			

¹ Subscript *USP* (e.g., Closure 5) indicates that the closure supplier’s classification was based on *USP* <381> tests performed according to *PF* 30(1) 2004. Otherwise, all suppliers’ classifications are based on *Ph. Eur.* 3.2.9 tests and criteria.

² Closure manufacturer stated Closure 13 is borderline Type II (some lots may fail).

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Row 1—*Specification*. Limits of Specification according to *USP* <381> final text.

Column 1—*Closure Random Identification Number*. The random identification code generated by PPI EC to blind the test sample population.

Column 2—*Final Type Classification*. The final classification (Type I or Type II) of the closure sample according to the closure manufacturer’s test results. (Note: If a closure fails to meet one or more of the Type I test requirements but still meets the Type II requirements for the test(s), then the closure merits a final classification of Type II.)

Column 3—*Test Specific Classification*. The classification (Type I or Type II) applies only for the specific test shown according to the closure manufacturer. For several

tests, typical data supplied by the closure manufacturer also may be included in this column (e.g., refer to *Table 7, Acidity and Alkalinity*).

Column 4—*Ph. Eur. Analyst 1*. These are data generated by WAL’s Analyst 1 according to *Ph. Eur.* 3.2.9.

Column 5—*USP Analyst 1*. These are data generated by WAL’s Analyst 1 according to *USP* <381> *PF* 30(1) 2004 with some modifications according to comments received.

Column 6—*USP Analyst 2*. These are data generated by WAL’s Analyst 2 according to *USP* <381> *PF* 30(1) 2004 with some modifications according to comments received.

Table 4. Appearance of Solution (Turbidity/Opalescence)—Procedure A (Visual)

Specification		Type I no more opalescent than <i>Reference Suspension B</i> (6 NTU) Type II no more opalescent than <i>Reference Suspension C</i> (18 NTU)				
Closure Random Identification Number	Closure Manufacturer–Supplied Information ¹		WAL Test Results			
	Final Type Classification	Test-Specific Classification	<i>Ph. Eur.</i> Analyst 1	<i>USP</i> Analyst 1	<i>USP</i> Analyst 2	
1	I	I	I	I	I	
3	I	I	I	I	I	
4	I	I	I	I	I	
6	I	I	I	I	I	
7	I	I	I	I	I	
10	I	I	I	I	I	
5	I _{USP} ¹	I _{USP}	I	I	I	
11	I _{USP}	I _{USP}	I	I	I	
2	II	I	I	I	I	
8	II	II	I, I	I, II	II	
9	II _{USP}	II _{USP}	I	I	I	
13	II _{USP} ²	I _{USP}	I	II	I	
12	Fail II	Fail II	I	II	I	

¹ Subscript *USP* (e.g., Closure 5) indicates that the closure supplier's classification was based on *USP* <381> tests performed according to *PF* 30(1) 2004. Otherwise, all suppliers' classifications are based on *Ph. Eur.* 3.2.9 tests and criteria.

² The closure manufacturer stated that Closure 13 is borderline Type II (some lots may fail).

Table 5. Appearance of Solution (Turbidity/Opalescence)—Procedure B (Instrumental)

Specification		Type I no more opalescent than Reference Suspension B (6 NTU) Type II no more opalescent than Reference Suspension C (18 NTU)				
Closure Random Identification Number	Closure Manufacturer–Supplied Information		WAL Test Results (NTU)			
	Final Type Classification ¹	Test-Specific Classification (NTU) ³	<i>Ph. Eur.</i> Analyst 1 ⁴	<i>USP</i> Analyst 1	<i>USP</i> Analyst 2	
1	I	n/a	n/a	I (0.5)	I (0.1)	
3	I	n/a	n/a	I (0.3)	I (0.2)	
4	I	n/a	n/a	I (0.3)	I (0.0)	
6	I	I (0.1 NTU according to ISO)	n/a	I (0.1)	I (0.1)	
7	I	I (0.0 NTU according to ISO)	n/a	I (0.2)	I (0.1)	
10	I	I (0.6 NTU according to ISO)	n/a	I (0.2)	I (0.3)	
5	I _{USP} ¹	I _{USP} (no NTU data supplied)	n/a	I (0.2)	I (0.2)	
11	I _{USP}	I _{USP} (no NTU data supplied)	n/a	I (0.2)	I (0.3)	
2	II	n/a	n/a	I (2.0)	I (1.5)	
8	II	II (<13.3 NTU according to ISO)	n/a	I (0.2) II (9.4)	II (7.7)	
9	II _{USP}	II _{USP} (no NTU data supplied)	n/a	II (7.7)	II (12.5)	

Table 5. Appearance of Solution (Turbidity/Opalescence)—Procedure B (Instrumental) (Continued)

Specification	Type I no more opalescent than Reference Suspension B (6 NTU) Type II no more opalescent than Reference Suspension C (18 NTU)				
Closure Random Identification Number	Closure Manufacturer–Supplied Information		WAL Test Results (NTU)		
	Final Type Classification ¹	Test-Specific Classification (NTU) ³	Ph. Eur. Analyst 1 ⁴	USP Analyst 1	USP Analyst 2
13	II _{USP} ²	I _{USP} (no NTU data supplied)	n/a	I (0.4)	I (0.3)
12	Fail II	n/a	n/a	II (6.2)	II (6.2)

¹ Subscript *USP* (e.g., Closure 5) indicates that the closure supplier’s classification was based on *USP* (381) tests performed according to *PF* 30(1) 2004. Otherwise, all suppliers’ final Type classifications are based on *Ph. Eur.* 3.2.9 tests and criteria.

² The closure manufacturer stated that Closure 13 is borderline Type II (some lots may fail).

³ Turbidity data are provided in NTU units. Instrumental turbidity tests were performed by suppliers either according to ISO 8871-1:2003(E), or according to *USP* (381) *PF* 30(1) 2004, as indicated. Otherwise, no instrumental data were provided (n/a).

⁴ Not applicable. No *Ph. Eur.* instrumental method was in place at the time of this study. Refer to *Table 4* for visual test Type classifications.

Table 6. Appearance of Solution (Color)

Specification	<i>Solution S</i> is not more intensely colored than <i>Color Standard</i>				
Closure Random Identification Number	Closure Manufacturer–Supplied Information ¹		WAL Test Results		
	Final Type Classification	Test-Specific Classification	Ph. Eur. Analyst 1	USP Analyst 1	USP Analyst 2
1	I	Pass	Pass	Pass	Pass
3	I	Pass	Pass	Pass	Pass
4	I	Pass	Pass	Pass	Pass
6	I	Pass	Pass	Pass	Pass
7	I	Pass	Pass	Pass	Pass
10	I	Pass	Pass	Pass	Pass
5	I _{USP} ¹	Pass _{USP}	Pass	Pass	Pass
11	I _{USP}	Pass _{USP}	Pass	Pass	Pass
2	II	Pass	Pass	Pass	Pass
8	II	Pass	Pass	Pass	Pass
9	II _{USP}	Pass _{USP}	Pass	Pass	Pass
13	II _{USP} ²	Pass _{USP}	Pass	Pass	Pass
12	Fail II	Pass	Pass	Pass	Pass

¹ Subscript *USP* (e.g., Closure 5) indicates that the closure supplier’s classification was based on *USP* (381) tests performed according to *PF* 30(1) 2004. Otherwise, all suppliers’ classifications are based on *Ph. Eur.* 3.2.9 tests and criteria.

² The closure manufacturer stated that Closure 13 is borderline Type II (some lots may fail).

Table 7. Acidity or Alkalinity

Specification		Not more than 0.3 mL 0.01 N NaOH Not more than 0.8 mL 0.01 N HCl			
Closure Random Identification Number	Closure Manufacturer–Supplied Information ¹		WAL Test Results		
	Final Type Classification	Test-Specific Classification	<i>Ph. Eur.</i> Analyst 1	<i>USP</i> Analyst 1	<i>USP</i> Analyst 2
1	I	Pass (0.0 mL)	Pass (0.7 mL acid)	Pass (0.1 mL base)	Pass (0.1 mL base)
3	I	Pass (0.04 mL base)	Pass (0.5 mL acid)	Pass (0.1 mL base)	Pass (0.0 mL)
4	I	Pass (0.04 mL base)	Pass (0.4 mL acid)	Pass (0.1 mL base)	Pass (0.1 mL base)
6	I	Pass (0.03 mL base)	Pass (0.8 mL acid)	Pass (0.2 mL base)	Pass (0.1 mL base)
7	I	Pass (0.0 mL)	Pass (0.7 mL acid)	Pass (0.2 mL base)	Pass (0.1 mL base)
10	I	Pass (0.03 mL base)	Pass (0.7 mL acid)	Pass (0.3 mL base)	Pass (0.0 mL)
5	<i>I</i> _{USP} ¹	Pass _{USP} (0.05 mL base)	Pass (0.8 mL acid)	Pass (0.1 mL base)	Pass (0.3 mL acid)
11	<i>I</i> _{USP}	Pass _{USP} (0.05 mL base)	Pass (0.7 mL acid)	Pass (0.1 mL base)	Pass (0.05 mL base)
2	II	Pass (0.10 acid)	Pass (0.8 mL acid)	Pass (0.0 mL)	Pass (0.1 mL base)
8	II	Pass (0.3 mL base)	Fail (1.1 mL acid)	Pass (0.1 mL base)	Pass (0.2 mL base)
9	<i>II</i> _{USP}	Pass _{USP} (0.68 mL acid)	Pass (0.75 mL acid)	Pass (0.1 mL base)	Pass (0.0 mL)
13	<i>II</i> _{USP} ²	Pass _{USP} (0.06 mL base)	Pass (0.7 mL acid)	Pass (0.2 mL base)	Pass (0.1 mL base)
12	Fail II	Pass (0.1 mL base)	Pass (0.8 mL acid)	Pass (0.1 mL acid)	Pass (0.0 mL)

¹ Subscript *USP* (e.g., Closure 5) indicates that the closure supplier's classification was based on *USP* <381> tests performed according to *PF* 30(1) 2004. Otherwise, all suppliers' classifications are based on *Ph. Eur.* 3.2.9 tests and criteria.

² The closure manufacturer stated that Closure 13 is borderline Type II (some lots may fail).

Table 8. Absorbance

Specification		Absorbance no more than 0.2 for Type I Absorbance no more than 4.0 for Type II				
Closure Random Identification Number	Closure Manufacturer–Supplied Information ¹		WAL Test Results			
	Final Type Classification	Test-Specific Classification	Ph. Eur. Analyst 1	USP Analyst 1	USP Analyst 2	
1	I	I (0.00)	I (0.05)	I (0.00)	I (0.05)	
3	I	I (≤0.2)	I (0.03)	I (0.05)	I (0.03)	
4	I	I (≤0.2)	I (0.02)	I (0.00)	I (0.00)	
6	I	I (0.01)	I (0.00)	I (0.00)	I (0.02)	
7	I	I (0.00)	I (0.00)	I (0.01)	I (0.00)	
10	I	I (0.01)	I (0.01)	I (0.01)	I (0.13)	
5	I _{USP} ¹	I _{USP} (0.02)	I (0.00)	I (0.01)	I (0.02)	
11	I _{USP}	I _{USP} (0.13)	I (0.08)	I (0.08)	I (0.06)	
2	II	II (1.3)	II (0.67)	II (0.50)	II (0.60)	
8	II	II (1.9)	II (0.55) II (1.4)	II (1.8) II (1.5)	II (1.5)	
9	I _{USP}	II _{USP} (2.8)	II (1.2)	II (1.9)	II (1.8)	
13	I _{USP} ²	II _{USP} (0.52)	II (0.53)	II (0.55)	II (0.47)	
12	Fail II	II (0.30)	II (0.22)	I (0.20)	I (0.19)	

¹ Subscript *USP* (e.g., Closure 5) indicates that the closure supplier's classification was based on *USP* <381> tests performed according to *PF* 30(1) 2004. Otherwise, all suppliers' classifications are based on *Ph. Eur.* 3.2.9 tests and criteria.

² The closure manufacturer stated that Closure 13 is borderline Type II (some lots may fail).

Table 9. Reducing Substances

Closure Random Identification Number	Closure Manufacturer–Supplied Information ¹		WAL Test Results (mL)		
	Final Type Classification	Test-Specific Classification	Ph. Eur. Analyst 1	USP Analyst 1	USP Analyst 2
1	I	I (1.0)	I (0.5)	I (0.8)	I (0.8)
3	I	I (0.1)	I (0.1)	I (0.0)	I (0.1)
4	I	I (0.1)	I (0.1)	I (0.1)	I (0.0)
6	I	I (0.05)	I (0.0)	I (0.0)	I (0.4)
7	I	I (0.5)	I (0.2)	I (0.0)	I (0.0)
10	I	I (0.1)	I (0.2)	I (1.0)	I (0.2)
5	I _{USP} ¹	I _{USP} (0.07)	I (0.1)	I (0.0)	I (0.5)
11	I _{USP}	I _{USP} (0.9)	I (1.2)	I (1.0)	I (1.0)
2	II	II (4.2)	I (2.0)	I (2.1)	I (2.5)
8	II	II (5.4)	I (2.2) II (3.3)	I (2.0) II (3.5)	II (3.3)
9	II _{USP}	I _{USP} (2.8)	I (2.3)	I (2.2)	II (4.7)
13	II _{USP} ²	I _{USP} (1.8)	II (3.2)	II (3.5)	I (1.2)
12	Fail II	II (5.6)	I (3.0)	I (2.6)	I (2.3)

¹ Subscript *USP* (e.g., Closure 5) indicates that the closure supplier's classification was based on *USP* <381> tests performed according to *PF* 30(1) 2004. Otherwise, all suppliers' classifications are based on *Ph. Eur.* 3.2.9 tests and criteria.

² The closure manufacturer stated that Closure 13 is borderline Type II (some lots may fail).

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Table 10. Heavy Metals

Specification		Solution S contains not more than 2 ppm heavy metals as Pb				
Closure Random Identification Number	Closure Manufacturer–Supplied Information ¹		WAL Test Results			
	Final Type Classification	Test-Specific Classification	Ph. Eur. Analyst 1	USP Analyst 1	USP Analyst 2	
1	I	Pass	Pass	Pass	Pass	
3	I	Pass	Pass	Pass	Pass	
4	I	Pass	Pass	Pass	Pass	
6	I	Pass	Pass	Pass	Pass	
7	I	Pass	Pass	Pass	Pass	
10	I	Pass	Pass	Pass	Pass	
5	I _{USP} ¹	Pass _{USP}	Pass	Pass	Pass	
11	I _{USP}	Pass _{USP}	Pass	Pass	Pass	
2	II	Pass	Pass	Pass	Pass	
8	II	Pass	Pass	Pass	Pass	
9	I _{USP}	Pass _{USP}	Pass	Pass	Pass	
13	II _{USP} ²	Pass _{USP}	Pass	Pass	Pass	
12	Fail II	Pass	Pass	Pass	Pass	

¹ Subscript *USP* (e.g., Closure 5) indicates that the closure supplier’s classification was based on *USP* <381> tests performed according to *PF* 30(1) 2004. Otherwise, all suppliers’ classifications are based on *Ph. Eur.* 3.2.9 tests and criteria.

² The closure manufacturer stated that Closure 13 is borderline Type II (some lots may fail).

Table 11. Extractable Zinc

Specification		Solution S contains not more than 5 ppm Zn				
Closure Random Identification Number	Closure Manufacturer–Supplied Information ¹		WAL Test Results			
	Final Type Classification	Test-Specific Classification	Ph. Eur. Analyst 1	USP Analyst 1	USP Analyst 2	
1	I	Pass	Pass	Pass	Pass	
3	I	Pass	Pass	Pass	Pass	
4	I	Pass	Pass	Pass	Pass	
6	I	Pass	Pass	Pass	Pass	
7	I	Pass	Pass	Pass	Pass	
10	I	Pass	Pass	Pass	Pass	
5	I _{USP} ¹	Pass _{USP}	Pass	Pass	Pass	
11	I _{USP}	Pass _{USP}	Pass	Pass	Pass	
2	II	Pass	Pass	Pass	Pass	
8	II	Pass	Pass	Pass	Pass	
9	I _{USP}	Pass _{USP}	Pass	Pass	Pass	
13	II _{USP} ²	Pass _{USP}	Pass	Pass	Pass	
12	Fail II	Pass	Pass	Pass	Pass	

¹ Subscript *USP* (e.g., Closure 5) indicates that the closure supplier’s classification was based on *USP* <381> tests performed according to *PF* 30(1) 2004. Otherwise, all suppliers’ classifications are based on *Ph. Eur.* 3.2.9 tests and criteria.

² The closure manufacturer stated that Closure 13 is borderline Type II (some lots may fail).

Stimuli to the Revision Process

Table 12. *Ammonium*

Specification		After 5 min, any yellow color in the <i>Test Solution</i> is no darker than the <i>Ammonium Standard Solution</i> (no more than 2 ppm NH ₄ in <i>Solution S</i>)			
Closure Random Identification Number	Closure Manufacturer–Supplied Information ¹		WAL Test Results		
	Final Type Classification	Test-Specific Classification	<i>Ph. Eur.</i> Analyst 1	<i>USP</i> Analyst 1	<i>USP</i> Analyst 2
1	I	Pass	Pass	Pass	Pass
3	I	Pass	Pass	Pass	Pass
4	I	Pass	Pass	Pass	Pass
6	I	Pass	Pass	Pass	Pass
7	I	Pass	Pass	Pass	Pass
10	I	Pass	Pass	Pass	Pass
5	I _{USP} ¹	Pass _{USP}	Pass	Pass	Pass
11	I _{USP}	Pass _{USP}	Pass	Pass	Pass
2	II	Pass	Pass	Pass	Pass
8	II	Pass	Pass	Pass	Pass
9	II _{USP}	Pass _{USP}	Pass	Pass	Pass
13	II _{USP} ²	Pass _{USP}	Pass	Pass	Pass
12	Fail II	Pass	Pass	Pass	Pass

¹ Subscript *USP* (e.g., Closure 5) indicates that the closure supplier's classification was based on *USP* <381> tests performed according to *PF* 30(1) 2004. Otherwise, all suppliers' classifications are based on *Ph. Eur.* 3.2.9 tests and criteria.

² The closure manufacturer stated that Closure 13 is borderline Type II (some lots may fail).

Table 13. *Volatile Sulfides*

Specification		Any black stain on the paper produced by <i>Solution S</i> is not more intense than that produced by the control solution.			
Closure Random Identification Number	Closure Manufacturer–Supplied Information ¹		WAL Test Results		
	Final Type Classification	Test-Specific Classification	<i>Ph. Eur.</i> Analyst 1	<i>USP</i> Analyst 1	<i>USP</i> Analyst 2
1	I	Pass	Pass	Pass	Pass
3	I	Pass	Pass	Pass	Pass
4	I	Pass	Pass	Pass	Pass
6	I	Pass	Pass	Pass	Pass
7	I	Pass	Pass	Pass	Pass
10	I	Pass	Pass	Pass	Pass
5	I _{USP} ¹	Pass _{USP}	Pass	Pass	Pass
11	I _{USP}	Pass _{USP}	Pass	Pass	Pass
2	II	Pass	Pass	Pass	Pass
8	II	Pass	Pass	Pass	Pass
9	II _{USP}	Pass _{USP}	Pass	Pass	Pass
13	II _{USP} ²	Pass _{USP}	Pass	Pass	Pass
12	Fail II	Pass	Pass	Pass	Pass

¹ Subscript *USP* (e.g., Closure 5) indicates that the closure supplier's classification was based on *USP* <381> tests performed according to *PF* 30(1) 2004. Otherwise, all suppliers' classifications are based on *Ph. Eur.* 3.2.9 tests and criteria.

² The closure manufacturer stated that Closure 13 is borderline Type II (some lots may fail).

Table 14. Final Elastomeric Closure Classification Comparison

Closure Random Identification Number	Closure Manufacturer's Final Type Classification ¹	USP Research Study Final Type Classification Results		
		Ph. Eur. Analyst 1	USP Analyst 1	USP Analyst 2
1	I	I	I	I
3	I	I	I	I
4	I	I	I	I
6	I	I	I	I
7	I	I	I	I
10	I	I	I	I
5	I _{USP} ¹	I	I	I
11	I _{USP}	I	I	I
2	II	II	II	II
8	II	II ³	II	II
9	II _{USP}	II	II	II
13	II _{USP} ²	II	II	II
12	Fail II	II	II	II

¹ Subscript *USP* (e.g., Closure 5) indicates that the closure supplier's classification was based on *USP* <381> tests performed according to *PF* 30(1) 2004. Otherwise, all suppliers' classifications are based on *Ph. Eur.* 3.2.9 tests and criteria.

² The closure manufacturer stated that Closure 13 is borderline Type II (some lots may fail).

³ Closure 8 failed the acidity/alkalinity test for 1 of 2 replicate tests performed (refer to *Table 7*).

APPENDIX

USP Elastomeric Closures for Injection <381>

Final Text as It Will Appear in *USP 31, 1st Supplement*
Scheduled to become official 1 August 2008

INTRODUCTION

Elastomeric closures for containers used in the types of preparations defined in General Chapter *Injections* <1> are made of materials obtained by vulcanization (cross-linking) polymerization, polyaddition, or polycondensation of macromolecular organic substances (elastomers). Closure formulations contain natural or synthetic elastomers and inorganic and organic additives to aid or control vulcanization, impart physical and chemical properties or color, or stabilize the closure formulation.

This General Chapter applies to closures formulated with natural or synthetic elastomeric substances. This chapter does not apply to closures made from silicone elastomer, but it does apply to closures treated with silicone (e.g., dimethicone, NF). The tests described in the present chapter do not require that closures be treated with silicone, although the procedures herein do not prohibit the use of siliconized closures.

The following comments relate solely to laminated or coated closures [e.g., polytetrafluoroethylene (PTFE) or lacquer coatings]. All *Physicochemical Tests* apply to the base elastomer of such closures. To obtain *Elastomeric Closures for Injections Physicochemical Tests* <381> results, analysts may perform the tests on uncoated or nonlaminated closures of the base elastomeric compound. The *Functionality Tests* apply to and should be performed using the entire laminated or coated elastomeric closure.

Biological Tests. Analysts may perform *Biological Tests* on the coating separately from the base elastomer or on the coated closure itself. For all *Elastomeric Closures for Injections* <381> tests performed on any closure type, analysts should document the closure under test, including a full description of the elastomer and any lubrication, coating, laminations, or treatments applied.

This chapter states test limits for Type I and Type II elastomeric closures. Type I closures are those used for aqueous preparations. Type II closures typically are intended for nonaqueous preparations. Type II closures are those that, because they have properties optimized for special uses, may not meet all requirements listed for Type I closures due to physical configuration, material of construction, or both. If a closure fails to meet one or more of the Type I test requirements but still meets the Type II requirements for the test(s), assign the closure a final classification of Type II.

This chapter is intended as an initial screen to identify elastomeric closures that on the basis of their biological compatibility, their aqueous extract physicochemical properties, and their functionality may be appropriate for use with injectable preparations. All elastomeric closures suitable for use with injectable preparations comply with either Type I or Type II test limits. However, this specification is not intended to serve as the sole evaluation criterion for the selection of such closures.

The following closure evaluation requirements are beyond the scope of this General Chapter:

- The establishment of closure identification tests and specifications
- The verification of closure–product physicochemical compatibility
- The identification and safety determination of closure leachables found in the packaged product
- The verification of packaged product closure functionality under actual storage and use conditions.

The manufacturer of the injectable product (the end user) must obtain from the closure supplier an assurance that the composition of the closure does not vary and that it is the same as that of the closure used during compatibility testing. When the supplier informs the end user of changes in the composition, the latter must repeat compatibility testing, totally or partly, depending on the nature of the changes. Closures must be properly stored, cleaned for removal of environmental contaminants and endotoxins, and sterilized prior to use in packaging injectable products.

Characteristics

Elastomeric closures are translucent or opaque and have no characteristic color (color depends on the additives used). They are homogeneous and practically free from flash and adventitious materials (e.g., fibers, foreign particles, and waste rubber.)

Identification

Vendors make closures from a wide variety of elastomeric materials and optional polymeric coatings. For this reason the present chapter does not attempt to specify identification tests that encompass all possible closure presentations. However, the closure supplier and the injectable product manufacturer (the end user) are responsible for verifying the closure elastomeric formulation and any coating or laminate materials used according to suitable identification tests. Examples of some of the analytical test methodologies that may be used include specific gravity, % ash analysis, sulfur content determination, Fourier Transform infrared spectrophotometry—attenuated total reflectance testing, thin-layer chromatography of an extract, ultraviolet absorption spectrophotometry of an extract, or infrared absorption spectrophotometry of a pyrolysate.

Test Procedures

Elastomeric closures shall conform to biological, physicochemical, and functionality requirements both as they are shipped by the closure supplier to the injectable product manufacturer (the end user) and in their final ready-to-use state by the end user.

For elastomeric closures that are processed by the supplier prior to distribution to the end user, the supplier shall demonstrate compendial conformance of closures exposed to such processing and/or sterilization steps. Similarly, if elastomeric closures received by the end user are subsequently processed or sterilized, the end user is responsible for demonstrating the continued conformance of closures to compendial requirements after such processing and/or sterilization (in other words, in their ready-to-use state). Careful attention to closures' continuing conformity is especially important if closures will be exposed to processes or conditions that may significantly influence their biological, physicochemical, or functionality characteristics (e.g., gamma irradiation).

For closures that normally are lubricated (e.g., siliconized) prior to use, analysts can perform physicochemical testing on nonlubricated closures in order to avoid potentially confounding variables introduced by lubricants, which may lead to interference with the procedure and/or difficulties in interpreting test results.

For laminated or coated closures (e.g., PTFE or lacquer coatings), physicochemical compendial tests apply to the noncoated base elastomer. In this case, suppliers are responsible for demonstrating physicochemical compendial compliance of the noncoated closure processed or treated in a manner simulating conditions that are followed for coated closures before shipment to the end user. End users of coated closures also are responsible for demonstrating the continued physicochemical compendial conformance of the base, uncoated elastomer following processing or sterilization in a manner that simulates the end-user's actual use conditions for the coated closures.

In all cases, document all conditions of closure processing, pretreatment, sterilization, or lubrication when reporting test results.

Biological Tests

Two stages of testing are indicated: The first stage is performance of the *in vitro* test procedure described in General Chapter *Biological Reactivity Tests, In Vitro* (87). Materials that do not meet the requirements of the *in vitro* test move to the second stage of testing, which includes the *in vivo* tests *Systemic Injection Test* and *Intracutaneous Test* set forth in General Chapter *Biological Reactivity Tests, In Vivo* (88). Materials that meet the requirements of the *in vitro* test do not require *in vivo* testing.

Type I and Type II closures must conform to the requirements of either the *in vitro* or the *in vivo* biological reactivity tests. [NOTE—Also see General Information Chapter *The Biocompatibility of Material Used in Drug Containers, Medical Devices, and Implants* (1031).]

Physicochemical Tests

Preparation of Solution S—Place whole, uncut closures corresponding to a surface area of $100 \pm 10 \text{ cm}^2$ into a suitable glass container. Cover the closures with 200 mL of *Purified Water* or *Water for Injection*. If the use of uncut closures will not achieve the prescribed closure surface area ($100 \pm 10 \text{ cm}^2$), select the number of closures that will most closely approximate 100 cm^2 , and adjust the volume of water used to the equivalent of 2 mL per each 1 cm^2 of actual closure surface area used. Boil for 5 minutes, and rinse five times with cold *Purified Water* or *Water for Injection*.

Place the washed closures into a Type I glass wide-necked flask (see *Containers* (661)), add a quantity of *Purified Water* or *Water for Injection* equivalent to that which was initially added to the closures, and weigh. Cover the mouth of the flask with a Type I glass beaker. Heat in an autoclave so that a temperature of $121 \pm 2^\circ$ is reached within 20 to 30 minutes, and maintain this temperature for 30 minutes. Cool to room temperature over a period of about 30 minutes. Add *Purified Water* or *Water for Injection* to bring it up to the original mass. Shake, and immediately decant and collect the solution. [NOTE—This solution must be shaken before being used in each of the tests.]

Preparation of Blank—Prepare a blank solution similarly, using 200 mL of *Purified Water* or *Water for Injection*, omitting the closures.

Appearance of Solution (Turbidity/Opaescence and Color)—

Determination of Turbidity (Opalescence)—[NOTE—Determine by visual comparison (*Procedure A*), or instrumentally using a suitable ratio turbidimeter (*Procedure B*)]. For a discussion of turbidimetry, see *Spectrophotometry and Light Scattering* (851). Instrumental assessment of clarity provides a more discriminatory test that does not depend on the visual acuity of the analyst.

Hydrazine sulfate solution—Dissolve 1.0 g of hydrazine sulfate in water and dilute to 100.0 mL with water. Allow to stand for 4 to 6 hours.

Hexamethylenetetramine solution—Dissolve 2.5 g of hexamethylenetetramine in 25.0 mL of water in a 100 mL glass-stoppered flask.

Opalescence Stock Suspension—Add 25.0 mL of hydrazine sulfate solution to the hexamethylenetetramine solution in the flask. Mix and allow to stand for 24 hours. This suspension is stable for 2 months, provided it is stored in a glass container that is free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.

Opalescence Standard Suspension—Prepare a suspension by diluting 15.0 mL of the *Opalescence Stock Suspension* with water to 1000.0 mL. *Opalescence Standard Suspension* is stable for about 24 hours after preparation.

Reference Suspensions—Prepare the *Reference Suspensions* according to *Table 1*. Mix and shake before use. [NOTE—Stabilized formazin suspensions that can be used to prepare stable, diluted turbidity standards are available commercially and may be used after comparison with the standards prepared as described.]

Table 1. Preparation of Reference Suspensions

	<i>Suspension A</i>	<i>Suspension B</i>	<i>Suspension C</i>	<i>Suspension D</i>
Standard of Opalescence	5.0 mL	10.0 mL	30.0 mL	50.0 mL
Water	95.0 mL	90.0 mL	70.0 mL	50.0 mL
Nephelometric Turbidity Units (NTUs)	3 NTU	6 NTU	18 NTU	30 NTU

Procedure A: Visual Comparison—Use identical test tubes made of colorless, transparent, neutral glass with a flat base and an internal diameter of 15 mm to 25 mm. Fill one tube to a depth of 40 mm with *Solution S*, one tube to the same depth with water, and four others to the same depth with *Reference Suspensions A, B, C, and D*. Compare the solutions in diffuse daylight 5 minutes after preparation of the reference suspensions, viewing vertically against a black background. The light conditions should be such that *Reference Suspension A* is readily distinguishable from water and that *Reference Suspension B* is readily distinguishable from *Reference Suspension A*.

Procedure A: Requirement—*Solution S* is not more opalescent than *Reference Suspension B* for Type I closures and not more opalescent than *Reference Suspension C* for Type II closures. *Solution S* is considered clear if its clarity is the same as

that of water when examined as described above or if its opalescence is not more pronounced than that of *Reference Suspension A* (*Table 2*).

Procedure B: Instrumental Comparison—Measure the turbidity of the *Reference Suspensions* in a suitable calibrated turbidimeter (see *Spectrophotometry and Light Scattering* (851)). Run the *blank* and correct the results. *Reference Suspensions A, B, C, and D* represent 3, 6, 18, and 30 Nephelometric turbidity units (NTU), respectively. Measure the turbidity of *Solution S* using the calibrated turbidimeter.

Procedure B: Requirement—The turbidity of *Solution S* is not greater than that for *Reference Suspension B* (6 NTU) for Type I closures and is not greater than that for *Reference Suspension C* (18 NTU) for Type II closures (*Table 2*).

Table 2. Opalescence Requirements

Opalescence Requirements	Comparison Method	
	Procedure A Visual	Procedure B Instrumental
Type I Closures	no more opalescent than <i>Suspension B</i>	no more than 6 NTU
Type II Closures	no more opalescent than <i>Suspension C</i>	no more than 18 NTU

Determination of Color—

Color Standard—Prepare a *Color Standard solution* by diluting 6.0 mL of *Matching Fluid O* (see *Color and Achromicity* (631)) with 94.0 mL of diluted hydrochloric acid.

Procedure—Use identical test tubes made of colorless, transparent, neutral glass with a flat base and an internal diameter of 15 mm to 25 mm. Fill one tube to a depth of 40 mm with *Solution S* and the second with *Color Standard*. Compare the liquids in diffuse daylight, viewing vertically against a white background.

Color Standard: Requirement—*Solution S* is not more intensely colored than the *Color Standard*.

Acidity or Alkalinity—

Bromothymol Blue Solution—Dissolve 50 mg of bromothymol blue in a mixture of 4 mL of 0.02 M sodium hydroxide and 20 mL of alcohol. Dilute with water to 100 mL.

Procedure—To 20 mL of *Solution S* add 0.1 mL of *Bromothymol Blue Solution*. If the solution is yellow, titrate with 0.01 N sodium hydroxide until it reaches a blue endpoint. If the solution is blue, titrate with 0.01 N hydrochloric acid until it reaches a yellow endpoint. If the solution is green, it is neutral and does not require titration.

Blank Correction—Test 20 mL of *blank* similarly. Correct the results obtained for *Solution S* by subtracting the volume of titrant required for *blank*. (Cf. *Titrimetry* (541).)

Acidity or Alkalinity: Requirement—Not more than 0.3 mL of 0.01 N sodium hydroxide produces a blue color, or not more than 0.8 mL of 0.01 N hydrochloric acid produces a yellow color, or no titration is required.

Absorbance—

Procedure—[NOTE—Perform this test within 5 hours of preparing *Solution S*.] Filter *Solution S* through a 0.45- μ m pore size filter, discarding the first few mL of filtrate. Measure the absorbance of the filtrate at wavelengths between 220 nm and 360 nm in a 1-cm cell using the *blank* in a matched cell in the reference beam. If dilution of the filtrate is required before measurement of the absorbance, correct the test results for the dilution.

Absorbance: Requirement—The absorbances at these wavelengths do not exceed 0.2 for Type I closures or 4.0 for Type II closures.

Reducing Substances—

Procedure—[NOTE—Perform this test within 4 hours of preparing *Solution S*.] To 20.0 mL of *Solution S* add 1 mL of diluted sulfuric acid and 20.0 mL of 0.002 M potassium permanganate. Boil for 3 minutes. Cool. Add 1 g of potassium iodide, and titrate immediately with 0.01 M sodium thiosulfate using 0.25 mL of starch solution TS as the indicator. Perform a titration using 20.0 mL of *blank*, and note the difference in volume of 0.01 M sodium thiosulfate required.

Reducing Substances: Requirement—The difference between the titration volumes is not greater than 3.0 mL for Type I closures and not greater than 7.0 mL for Type II closures.

Heavy Metals—

Procedure—Proceed as directed for *Method 1* under *Heavy Metals* (231). Prepare the *Test Preparation* using 10.0 mL of *Solution S*.

Heavy Metals: Requirement—*Solution S* contains not more than 2 ppm heavy metals as lead.

Extractable Zinc—

Test Solution—Prepare a *Test Solution* by diluting 10.0 mL of *Solution S* to 100 mL with 0.1 N hydrochloric acid. Prepare a test *blank* similarly, using the *blank* for *Solution S*.

Zinc Standard Solution—Prepare a *Zinc Standard Solution* (10 ppm Zn) by dissolving zinc sulfate in 0.1 N hydrochloric acid.

Reference Solutions—Prepare not fewer than 3 *Reference Solutions* by diluting the *Zinc Standard Solution* with 0.1 N hydrochloric acid. The concentrations of zinc in these *Reference Solutions* should span the expected limit of the *Test Solution*.

Procedure—Use a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light Scattering* (851)) equipped with a zinc hollow-cathode lamp and an air-acetylene flame. An alternative procedure such as an appropriately validated inductively coupled plasma (ICP) analysis is suitable. Test each of the *Reference Solutions* at the zinc emission line 213.9 nm at least 3 times. Record the steady readings. Rinse the apparatus with the test blank solution each time to ensure that the reading returns to the initial blank value. Prepare a calibration curve from the mean of the readings obtained for each *Reference Solution*. Record the absorbance of the *Test Solution*. Determine the *Test Solution* ppm zinc concentration using the calibration curve.

Requirement—*Solution S* contains not more than 5 ppm of extractable zinc.

Ammonium—

Alkaline Potassium Tetraiodomercurate Solution—Prepare a 100-mL solution containing 11 g of potassium iodide and 15 g of mercuric iodide in water. Immediately before use mix 1 volume of this solution with an equal volume of a 250 g/L solution of sodium hydroxide.

Test Solution—Dilute 5 mL of *Solution S* to 14 mL with water. Make alkaline if necessary by adding 1 N sodium hydroxide, and dilute to 15 mL with water. Add 0.3 mL of *Alkaline Potassium Tetraiodomercurate Solution*. Close the container.

Ammonium Standard Solution—Prepare a solution of ammonium chloride in water (1 ppm NH_4). Mix 10 mL of the 1 ppm ammonium chloride solution with 5 mL water and 0.3 mL of *Alkaline Potassium Tetraiodomercurate Solution*. Close the container.

Ammonium: Requirement—After 5 minutes, any yellow color in the *Test Solution* is no darker than the *Ammonium Standard Solution* (no more than 2 ppm NH_4 in *Solution S*).

Volatile Sulfides—

Procedure—Place closures, cut if necessary, with a total surface area of 20 ± 2 cm² in a 100 mL flask, and add 50 mL of a 20 g/L citric acid solution. In the same manner and at the same time, prepare a control solution in a separate 100 mL flask by dissolving 0.154 mg of sodium sulfide in 50 mL of a 20 g/L citric acid solution. Place a piece of lead acetate paper over the mouth of each flask, and hold the paper in position by placing over it an inverted weighing bottle. Heat the flasks in an autoclave at $121 \pm 2^\circ$ for 30 minutes.

Volatile Sulfides: Requirement—Any black stain on the paper produced by *Solution S* is not more intense than that produced by the control solution.

Functionality Tests

NOTE—Samples washed as described for preparation of *Solution S* (i.e., boiled and rinsed) and subsequently air dried are used for *Functionality Tests of Penetrability, Fragmentation, and Self-sealing Capacity*. *Functionality Tests* are appropriate for closures that will be pierced by a hypodermic needle. *Self-sealing Capacity* is required only for closures intended for use with multiple-dose containers. The needle specified for each test is a lubricated long-bevel (bevel angle $12 \pm 2^\circ$) hypodermic needle¹ with an external diameter of 0.8 mm (21 Gauge).

Penetrability—

Procedure—Fill 10 suitable vials to the nominal volume with water, fit the closures that will be examined, and secure with a cap. Using a new hypodermic needle as described above for each closure, pierce the closure with the needle perpendicular to the surface.

Penetrability: Requirement—The force for piercing is no greater than 10 N (1 kgf) for each closure, determined with an accuracy of ± 0.25 N (25 gf).

Fragmentation—

Closures for Liquid Preparations—Fill 12 clean vials with water to 4 mL less than the nominal capacity. Fit the closures to be examined, secure with a cap, and allow to stand for 16 hours.

Closures for Dry Preparations—Fit closures to be examined into 12 clean vials, and secure each with a cap.

Procedure—Using a hypodermic needle as described above fitted to a clean syringe, inject into each vial 1 mL of water while removing 1 mL of air. Repeat this procedure 4 times for each closure, piercing each time at a different site. Use a new needle for each closure, checking that it is not blunted during the test. Filter the total volume of liquid in all the vials through a single filter with porosity no greater than 0.5 μm . Count the rubber fragments visible to the naked eye on the surface of the filter.

Fragmentation: Requirement—No more than 5 fragments are visible. This limit is based on the assumption that fragments with a diameter $>50 \mu\text{m}$ are visible to the naked eye. In case of doubt or dispute, examine the particles microscopically to verify their nature and size.

Self-Sealing Capacity—

Procedure—Fill 10 suitable vials with water to the nominal volume. Fit the closures that are to be examined, and cap. Using a new hypodermic needle as described above for each closure, pierce each closure 10 times, piercing each time at a different site. Immerse the 10 vials in a solution of 0.1% (1 g/L) methylene blue, and reduce the external pressure by 27 kPa for 10 minutes. Restore to atmospheric pressure, and leave the vials immersed for 30 minutes. Rinse the outside of the vials.

Self-Sealing Capacity: Requirement—None of the vials contain any trace of blue solution.

¹ See ISO 7864, Sterile Hypodermic Needles for Single Use.